

Lymphatic Absorption of Seal and Fish Oils and Their Effect on Lipid Metabolism and Eicosanoid Production in Rats

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Eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) were distributed mainly in the sn-1 and 3 positions of seal oil triglyceride and in the sn-2 position of fish oil triglyceride. In Expt. 1, the structural distribution of EPA and DHA in lymph triglyceride of rats given seal or fish oils was similar to the distribution in the administered oils. In Expt. 2, seal oil-rich or fish oil-rich fats having constant polyunsaturated/monounsaturated/saturated fatty acids and n-6/n-3 polyunsaturated fatty acids ratios were fed to rats for 3 weeks. Seal oil more effectively reduced plasma and liver triglyceride than fish oil. Ratio of the productions of aortic prostacyclin and platelet thromboxane A₂ stimulated by thrombin was significantly higher in rats fed seal oil than in those fed fish oil. The results suggested that the different intramolecular distribution of EPA and DHA in dietary fat affected lipid metabolism differently in rats.

Key words: fish oil; lipid metabolism; prostacyclin; seal oil; thromboxane

Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids contained in marine oils are known to have several important physiological and pharmacological functions.¹⁻⁵⁾ Most studies of EPA and DHA have been done with fish oils in which these n-3 polyunsaturated fatty acids (PUFAs) are mainly at the second position (sn-2) in the triglyceride. Epidemiological studies in Greenland Inuit who live on mammals such as seals found a low rate of coronary heart diseases.⁶⁾ EPA and DHA in seal triglyceride are known to be mainly at the 1 (sn-1) and 3 (sn-3) positions. There are several studies showing that differences in intramolecular distribution of fatty acids in triglyceride may influence physiological functions of the fatty acids.⁷⁻¹⁰⁾ However, these studies were mainly done using vegetable and animal oils. Although Christensen *et al.* recently reported that metabolism of chylomicrons originated from dietary seal oil was different from that from fish oil,¹¹⁾ little information is available on the effects of the intramolecular structure of triglycerides rich in EPA and DHA on lipid metabolism.

It is reported that EPA and DHA at the 1 and 3 positions of triglyceride are resistant to hydrolysis by pancreatic lipase¹²⁾ and have a lower absorption rate.¹³⁾ However, we observed that EPA and DHA given as triicosapentaenoyl glycerol and tridocosahexaenoyl glycerol did not show lower absorbability, although the hydrolysis of these triglycerides by pancreatic lipase was slow.¹⁴⁾ Christensen *et al.* observed slower recovery in mesenteric lymph of EPA and DHA given as seal oil compared to fish oil.¹⁵⁾ Therefore, the influence of the location of EPA and DHA in triglyceride on their lymphatic absorption is still obscure. Although it is known that fatty acid at the 2 position of dietary triglyceride is well conserved during absorption processes,¹⁶⁾ there is little data on the positional distribu-

tion of EPA and DHA in lymph triglyceride after oral administration of seal or fish oils.

In this study, lymphatic transport and physiological effects of dietary seal and fish oils on lipid metabolism were examined in rats.

Materials and Methods

Materials. Seal oil was from harp seals. Fish oil was a mixture of orbital fat of tuna and sardine oils. These oils were purified to edible oil grade. The peroxide values of seal and fish oils were 8.6 and 6.9, respectively. The fatty acid compositions of fish and seal oils and intramolecular distribution of the fatty acids are shown in Table I. Palm oil was provided by Fuji Oil, Osaka. High oleic safflower oil and safflower oil were provided by Rinoru Oil Mills, Tokyo. Fatty acid-free bovine serum albumin fraction V was obtained from Miles Inc., Kankakee IL, U.S.A. Sodium taurocholate (purity >98%) was from Nacalai Tesque, Kyoto.

Experiment 1. Male Sprague-Dawley rats, weighing about 308 g (Seiwa Experimental Animals, Fukuoka), were fed on a non-purified diet (Type NMF, Oriental Yeast Co., Tokyo) and given drinking water *ad libitum* for 5 days. Under nembutal anesthesia, a cannula was inserted into the left thoracic channel of each rat for collecting lymph and also a catheter was put into the stomach.^{14,17)} After the surgery, rats were placed in restraining cages in a warm recovery room. They were given a normal osmotic solution containing 139 mm glucose and 85 mm NaCl as drinking water, and it was infused at a rate of 3 ml/h *via* the gastric tube until the end of this experiment. Next morning, after collection of 2 h lymph for a blank, 3 ml of emulsified seal or fish oil was administered to the rats *via* the gastric tube. The emulsions contained 200 mg seal or fish oil, 50 mg fatty acid-free bovine serum albumin, and 200 mg sodium taurocholate were prepared by sonication. After the administration of the emulsion, lymph was collected in a tube containing EDTA at 3 h intervals until 9 h at a single collection from 9 to 24 h.

Experiment 2. Male Sprague-Dawley rats, 4 weeks old weighing about 129 g (Seiwa Experimental Animals, Fukuoka), were divided into 3 groups of six animals. The rats were kept in an air-conditioned room (21-24°C, light on 08:00-20:00). The experimental diets were prepared according to the recommendation of the American Institute of Nutrition,¹⁸⁾ and

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Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; VLDL, very low density lipoprotein; TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

Table I. Fatty Acid Composition of Fish and Seal Oils

Fatty acids	Fish oil			Seal oil		
	Total	sn-2	sn-1,3	Total	sn-2	sn-1,3
	mol%					
14:0	4.9	3.2	4.2	4.0	6.1	1.9
14:1	0.5	0.0	0.4	3.3	5.1	1.2
16:0	10.4	8.4	11.3	1.8	3.5	1.5
16:1	11.3	8.5	12.4	18.4	35.2	11.1
16:2	2.3	3.3	2.9	1.6	3.1	1.2
16:3	1.5	1.4	2.0	1.0	1.3	1.0
16:4	2.1	2.2	1.9	1.8	1.9	1.8
18:0	1.9	0.9	2.6	0.1	0.2	0.2
18:1(n-9)	12.8	8.5	16.3	13.7	20.8	11.9
18:1(n-7)	3.8	2.2	5.1	2.2	2.4	2.2
18:2(n-6)	1.5	1.3	1.7	2.5	6.4	1.0
18:3(n-3)	1.1	1.0	1.3	1.6	3.7	0.9
18:4(n-3)	4.4	4.7	4.3	6.2	4.2	7.7
20:1	2.4	2.2	3.3	3.0	0.8	4.0
20:4(n-6)	1.5	1.8	1.3	0.5	0.1	0.6
20:4(n-3)	0.8	0.8	1.0	1.0	0.5	1.3
20:5(n-3)	18.5	20.7	17.3	14.1	2.1	20.3
22:1(n-9)	0.9	0.9	1.3	0.6	0.0	0.8
22:5(n-3)	1.5	3.0	1.1	5.7	0.7	8.7
22:6(n-3)	16.0	25.0	8.5	16.8	1.7	20.9

Table II. Fatty Acid Composition of Dietary Fats

Fatty acids	Groups		
	Control	Fish oil	Seal oil
	wt%		
14:0	0.7	1.1	1.0
16:0	28.9	26.6	26.9
16:1	0.2	1.8	2.8
18:0	3.3	3.1	3.1
18:1(n-9)	32.3	29.7	28.6
18:2(n-6)	32.1	22.8	23.5
18:4(n-3)	0.0	0.8	1.0
20:5(n-3)	0.0	3.6	2.7
22:5(n-3)	0.0	0.3	1.2
22:6(n-3)	0.0	3.5	3.6
SFA	34.5	34.5	34.1
MUFA	33.0	33.3	33.3
PUFA(n-6)	32.1	23.3	23.5
PUFA(n-3)	0.5	9.0	9.1

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Fatty acids less than 1% were not shown.

contained (g/kg diet) cornstarch 367, casein 200, α -cornstarch 132, sucrose 100, fat 100, cellulose 50, mineral mixture (AIN-93G-MX) 35, vitamin mixture (AIN-93G-VX) 10, L-cystine 3, choline bitartrate 2.5, and *tert*-butylhydroquinone 0.014. The mineral and vitamin mixtures were purchased from Nihon Nosan Kogyo, Tokyo. Dietary fats were designed to have a constant polyunsaturated/monounsaturated/saturated fatty acid ratio of 1/1/1. Dietary fats in the control group were a mixture of palm, high oleic safflower, and safflower oils (56.3/7.7/36.0, by weight). Dietary fats in the groups of seal and fish oils were a mixture of seal, palm, high oleic safflower, and safflower oils (20.9/54.4/0.9/23.8) and fish, palm, high oleic safflower, and safflower oils (21.8/48.7/6.4/23.1), respectively. The fatty acid compositions of the three dietary fats are shown in Table II. Seal and fish oils contained cholesterol at less than 0.7%. Therefore, cholesterol contents in the diets containing seal or fish oils were less than 0.015%. Only a freshly prepared experimental diet was given to rats in every evening for 3 weeks. After they were starved for 7 h (7:00–14:00), the rats were killed by withdrawing blood from abdominal aorta in a syringe containing 3.8% trisodium citrate under diethyl ether anesthesia.

Analysis of lipids. Triglyceride structures of seal and fish oils and lymph triglyceride were analyzed by a Grignard degradation method.^{19–21} Ethyl gallate (25 mg/liter in solution) was added in solvents as an antioxidant.²² Oil (10 mg as triglyceride) was dissolved in 0.6 ml of diethyl ether. Under continuous mixing with a magnetic stirrer, 0.25 ml of 1 M ethylmagnesium bromide in diethyl ether (Aldrich Chemical Co., Milwaukee, WI, U.S.A.) was added. After 25 s, 0.25 ml of acetic acid–diethyl ether (1:9, v/v) was added to stop the reaction and 5 ml of 10% boric acid solution were added while mixing. The reaction mixture was extracted by 5 ml of diethyl ether saturated with 10% boric acid. The diethyl ether phase was washed twice with 1 ml of 2% aqueous NaHCO_3 and twice with 1 ml water. The solution was dried by a small amount of anhydrous sodium sulfate and dried up under nitrogen at room temperature. The lipids were applied to a silica gel G thin layer chromatography (TLC) plate containing 5% boric acid and the plate was developed with chloroform–acetone (96:4, v/v). 2-Monoglyceride and 1,3-diglyceride fractions were transmethylated with sulfuric acid–methanol (1:115, v/v). Fatty acid methyl esters extracted with hexane were analyzed by gas-liquid chromatography (GLC) on an Omegawax 320 capillary column (Supelco Japan, Tokyo; 30 meters length, 0.25 mm film, 0.32 mm i.d., helium as a carrier gas, and split ratio of 1:100). Column, injector, and detector temperatures were 200°C, 250°C, and 250°C, respectively.

Lipids were extracted by the method of Folch *et al.*²³ Cholesterol,²⁴ triglyceride,²⁵ and phospholipid²⁶ in liver were analyzed as previously described. Plasma cholesterol, triglyceride, and phospholipid were measured using a cholesterol C-test Wako, triglyceride G-test Wako, and

phospholipid B-test Wako (Wako Pure Chemical Industries, Osaka), respectively. Plasma high density lipoprotein (HDL-cholesterol) was analyzed with HDL-C-DAIICHI (Daiichi Pure Chemicals, Tokyo). Plasma and tissue lipids were separated into triglyceride and phospholipids by TLC and the fatty acid composition was analyzed by GLC as described. Pentadecanoic acid (Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as an internal standard for measurement of fatty acids in lymph lipids. The fatty acid recovery rate was calculated by subtracting the fatty acid amount in the blank lymph from that in the lymph collected after the administration of the test emulsion.

Analysis of prostaglandin and platelet aggregation. 6-Keto-prostaglandin $\text{F}_{1\alpha}$ and thromboxane B_2 (TXB_2) were analyzed as stable metabolites of prostacyclin (PGI_2) and thromboxane A_2 (TXA_2), respectively. Approximately 28 mg of thoracic aorta was incubated in Krebs–Henseleit bicarbonate buffer (pH 7.4) at 25°C for 30 min,²⁷ and the concentration of 6-keto-prostaglandin $\text{F}_{1\alpha}$ was measured by using a 6-keto-prostaglandin $\text{F}_{1\alpha}$ enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). Citrated plasma was centrifuged at $160 \times g$ for 10 min to obtain platelet-rich plasma and platelet-poor plasma was obtained from centrifugation of platelet-rich plasma at $1000 \times g$ for 15 min. TXB_2 concentration in platelet-rich plasma, platelet-rich plasma stimulated by ADP (final concentration of ADP was $5 \mu\text{M}$), and citrated blood stimulated by thrombin (final concentration of thrombin was 1 U/200 μl citrated blood) was measured by using a TXB_2 enzyme immunoassay kit (Cayman Chemical Co.). Platelet aggregation induced by $5 \mu\text{M}$ ADP was measured with an automated platelet aggregation analyzer (Aggrecorder II, Kyoto Daiichi Kagaku, Kyoto).²⁸

Statistical analysis. Data were analyzed by Student's *t*-test²⁹ in Expt. 1 and Duncan's new multiple-range test³⁰ in Expt. 2. Differences were considered significant at $p < 0.05$.

Results

Experiment 1

Lymphatic recovery of fatty acids. There was no difference in lymph flow rate between rats given seal and fish oils (Table III). Lymphatic recoveries for 24 h of EPA and DHA in rats given fish oil were significantly higher than those in rats given seal oil, although the recoveries until 9 h were the same between the two fats (Fig.). There was no significant difference in the recovery of fatty acids other than EPA and DHA.

Structural distribution of fatty acids in lymph triglyceride. EPA and DHA and 22:5n-3 in seal oil were mainly located